Reference: *Biol. Bull.* **175**: 261–273. (October, 1988)

Visual Spectral Sensitivities of Bioluminescent Deep-sea Crustaceans

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Abstract. The spectral sensitivities of eight species of deep-sea decapod shrimps (Family Oplophoridae) were determined from shipboard measurements of electroretinograms of dark-captured specimens. *Notostomus gibbosus* and *N. elegans* are maximally sensitive at 490 nm, and chromatic adaptation experiments indicate that a single visual pigment is present. Peak sensitivities of *Acanthephyra smithi* and *A. curtirostris* are at 510 nm, a longer wavelength than expected for such deep-sea dwellers. The four photophore-bearing species, *Systellaspis debilis, Janicella spinacauda, Oplophorus spinosus,* and *O. gracilirostris* have sensitivity maxima at 400 and 500 nm, and chromatic adaptation experiments indicate the presence of two visual pigments. This unusual short wavelength sensitivity may provide the basis for congener recognition based on the spectral bandwidth of luminescence.

Introduction

The light field in the deep-sea consists of essentially monochromatic light from two sources: (1) dim downwelling light with a chromatic spectrum centering on 475 nm (Jerlov, 1968; Dartnall, 1974; Cronin, 1986); and (2) bioluminescence, with spectra characteristically peaking at 460–490 nm (Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988). It has long been assumed that the visual systems of deep-sea organisms would also have monochromatic sensitivity, with visual pigment absorption maxima blue-shifted (as compared with shallow water species) for maximum sensitivity to the existing light regime (Bayliss *et al.*, 1936; Clarke, 1936; Goldsmith, 1972; Shaw and Stowe, 1982; Cronin, 1986). Almost all studies on deep-sea fish (Denton and Warren, 1957; Munz, 1957; Wald *et al.*, 1957; Denton and Shaw, 1963; Fernandez, 1978; Crescitelli *et al.*, 1985), cephalopods (Hara and Hara, 1979) and crustaceans (Fisher and Goldie, 1958, 1960; Denys and Brown, 1982) support this hypothesis, reporting single visual pigment systems with absorption maxima between 470 and 490 nm, in contrast with maxima of 490–550 for shallow water species (reviewed by Goldsmith, 1972; Lythgoe, 1972). However, these studies were performed on visual pigment extracts or *via* microspectrophotometry (MSP), which provide excellent information on the absorption characteristics of the visual pigments (see Menzel, 1979, for review of problems associated with extracts of invertebrate visual pigments), but may not necessarily reflect the physiological spectral sensitivity. For example, in the crayfish, *Procambarus,* the spectral sensitivity of the dark-adapted eye, measured electrophysiologically, peaks at about 570 nm (Wald, 1968), while the λ_{max} of the visual pigment is 530 nm; (Cummins and Goldsmith, 1981). Similar, though smaller, red shifts in spectral sensitivity are also found in the lobster, *Homarus,* (Wald and Hubbard, 1957; Wald, 1968) and the shrimp, *Palaemonetes,* (Fernandez, 1965; Wald and Seldin, 1968; Goldsmith and Fernandez, 1968). Goldsmith (1978) found that these shifts could be attributed to the filtering effects of red-leaky screening pigments. It is difficult, from pigment extract and MSP data, to accurately assess the degree of pre-retinal filtering and its effect on spectral sensitivity, but this must be taken into account before making any assessment of an organism's visual capacity.

Recording the electroretinogram, the mass response of a large number of photoreceptor cells to a flash of light, is a simple way to obtain physiologically relevant information about an organism's visual capabilities. For our

Received 24 December 1987; accepted 28 July 1988.

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purposes, it is superior to intracellular recording methods because of the necessity of working on a vibrating, unstable ship. Although data from this method cannot definitively establish whether one or several visual pigments are present, there is excellent evidence that ERG-determined peaks correspond to spectral cell types (Goldsmith and Fernandez, 1968; Stieve *et al.*, 1978; Laughlin *et al.*, 1980; Cummins and Goldsmith, 1981; Goldsmith, 1986) and this method is often the method of choice for comparative studies, particularly on previously untested organisms (Kobayashi and Ali, 1971; Eguchi *et al.*, 1982).

The first evidence for a short wavelength receptor in a deep-sea organism comes from Wald and Rayport's (1977) electrophysiological study of the alciopid worm *Vanadis*. Its dark-adapted spectral sensitivity curve exhibits a violet shoulder in addition to a blue-green peak, and response waveforms to 380 nm light are different from those to 480 nm light, arguing for the presence of two spectral classes of photoreceptor cells.

The fact that this is the only example of enhanced short wavelength sensitivity in deep-sea organisms may be because so few animals from this environment have been studied electrophysiologically. While both pigment extracts and MSP have proven successful in identifying dual red-shifted pigments in deep-sea fish (Denton *et al.*, 1970; O'Day and Fernandez, 1974; Partridge *et al.*, 1987), neither method has led to conclusive identification of the violet visual pigment (whose presence was verified with intracellular recordings) of some shallow water crustaceans (Goldsmith *et al.*, 1968; Goldsmith and Bruno, 1973; Cummins and Goldsmith, 1981; Martin and Mote, 1982; Cummins *et al.*, 1984). This may be due to the small quantity of pigment present, which would be swamped by the dominant pigment or its photoproducts during absorption measurements on extracts, or due to its location in small cells which may be inaccessible to, or overlooked by, MSP measurements.

Deep-sea crustaceans are useful subjects for exploratory electrophysiological studies of receptor systems of deep-sea animals, because they can be retrieved in good condition, and remain viable for many days under the proper maintenance conditions. Soft-bodied fish and invertebrates are generally dead upon retrieval or die within hours of capture. However, electrophysiological techniques have seldom been used with deep-sea crustaceans because most they rarely survive transport to land-based labs in good condition. For this reason, we have developed a portable electrophysiological apparatus enabling shipboard measurements of spectral sensitivities of freshly caught specimen. Members of the family Oplophoridae were chosen for this study because (1) this family contains both photophore bearing and non-photophore bearing species, (2) their depth ranges are fairly well known, and (3) viable specimens could be obtained in sufficient numbers for a comprehensive study. An unexpected result of this work was the discovery of enhanced sensitivity to violet light in the four photophore bearing species examined. Preliminary reports have been presented in abstract form (Frank, 1986).

Materials and Methods

Specimen collection and maintenance

Specimens of the eight species of deep-sea shrimp used in this study (Table 1) were collected during two cruises on the R.V. *New Horizon* off the southwest coast of Oahu, Hawaii, with an opening/closing 3.1 m Tucker Trawl, fitted with a thermally protected, light-tight collecting container (Childress *et al.*, 1977; Childress and Price, 1978). This container was closed at depth, ensuring recovery of healthy organisms whose eyes had not been irreparably damaged by surface light levels, a well known concern in working with deep-sea species (Loew, 1976; Nilsson and Lindstrom, 1983; Shelton *et al.*, 1985). The container was opened in a light tight room, and animals were sorted under dim red light. Experimental animals were maintained in chilled seawater (5°C) in light proof containers and studied within 24 hours of capture.

Dim red illumination was also used while setting up for experiments. Animals were mounted in a holder suspended in a 5°C seawater bath, allowing enough pleopod movement to maintain respiratory water currents across the gills. Temperature was maintained by pumping −1°C antifreeze from a Lauda cooler through cooling coils submerged in the seawater bath. The eyes were stabilized by gluing (Superglue) to small posts attached to the holding chamber on either side of the head.

Electrical recording

ERGs were recorded with 5 μm tip, glass insulated, metal microelectrodes (F. Haer & Co.), placed subcorneally with the aid of a dissecting microscope equipped with an infrared light source (Wratten Filter 89C) and an infrared image converter (FJW Industries). A reference electrode was placed in the other eye, and a silver-chloride electrode grounded the water bath. The electrodes were used with a Grass high impedance probe (Model HIP511, 10^7 *M* ohms impedance) to eliminate electrode polarization artifacts (Kugel, 1977). Signals were amplified with a Grass AC Pre-amplifier (Model HIP511J), with the low frequency filter set for minimal filtering (0.1–0.3 Hz) to minimize distortion due to AC-amplification.

Optical apparatus

Monochromatic test flashes were provided by an American ISA Monochromator (full width at half maximum intensity [FWHM] = 2 nm) with a tungsten - halogen light source powered by a Weston regulated power supply (Model 7521). Flash duration of 100 ms was controlled by a Uniblitz Shutter (Model 100-2) triggered by a Grass S44 Stimulator. Light intensity was controlled with a neutral density wedge and neutral density filters, and was calibrated at each wavelength with a UDT Optometer (United Detector Technology Model 61) and radiometric probe, with point calibrations referenced to NBS provided by UDT.

Test flashes were presented to the eye through one end of a branched quartz fiber optic light guide (Welch-Allen). The 2 mm output diameter of the light guide was large enough to illuminate the whole eye, and experiments showed that this light did not reach the reference eye.

The adapting light source for chromatic adaptation experiments was an incandescent light filtered by a 400 nm broadband filter (Melles Griot BG12, FWHM = 110) for violet adaptation, and a 520 nm broadband filter (M. G. VG6, FWHM = 90 nm) for green adaptation. The adapting light was delivered to the eye through one branch of the light guide, and test flashes were superimposed on this background light through the other branch. This ensured that both the adapting light and the stimulus light acted upon the same group of photoreceptor cells.

Experimental procedure

The eye was stimulated with 100 ms test flashes of monochromatic light adjusted for intensity until a defined criterion response was attained at each wavelength tested. The criterion was usually set 20 μV above baseline noise, ensuring that the intensity of the light flashes used was very near the threshold of sensitivity, so as not to light-adapt the eye. Signals were instantaneously analysed for peak to peak response height after digital conversion by an LSI/PDP 11 computer, and stored on magnetic tape (Lockheed Store 4 Recorder) for later waveform analysis. The order of the flashes was random, and the response to a standard flash of set wavelength and intensity was tested periodically throughout the experiment, to ensure the stability of the eye and to monitor the state of dark-adaptation. Spectral sensitivity measurements were started when the response to the standard flash was stable, for both dark-adapted and chromatically adapted eyes. Spectral sensitivity curves were generated as the reciprocal quanta needed to produce the criterion response at each wavelength. Absorptance spectra were constructed from Dartnall nomograms (Dartnall, 1953), using the analysis provided by Cornwall *et al.* (1984).

The inefficiency of the monochromator at shorter wavelengths limited the intensity of the adapting light that could be used. To ensure that a full spectral sensitivity curve could be measured, the intensity of the adapting light was adjusted so that a criterion response to 370 nm test flashes could still be elicited. Due to the varying sensitivity of some species to short wavelength light, the intensity of the adapting light necessarily varied between experiments.

Results

Notostomus gibbosus *and* N. elegans

The results for *N. gibbosus* and *N. elegans* were identical and will therefore be described together with no distinction made between species. The mean dark-adapted spectral sensitivity curve for *Notostomus* (Fig. 1A) shows that the sensitivity maximum occurred at about 490 nm. The absorptance spectrum for a 490 nm pigment with an optical density (O.D.) of .5 provides an excellent fit to the spectral sensitivity curve. Green chromatic adaptation uniformly depressed sensitivity across the spectrum (Fig. 1B), indicating that only one visual pigment is present in both species.

The dark-adapted response waveforms of the ERGs were identical at all wavelengths (Fig. 1C). Chromatic adaptation of the eyes with green light produced no discernible effects on the waveforms, supporting the conclusion that both species possess a single visual pigment.

Acanthephyra smithi *and* A. curtirostris

Although these two species have different depth distributions (Table 1), their spectral sensitivities were identical, and will again be described with no distinction between species. Maximum sensitivity in the dark-adapted eye was at 510 nm (Fig. 2A). An absorptance spectrum, constructed based on the known absorption maximum (490 nm) and O.D. (.6) of *A. smithi* visual pigment (Hiller-Adams *et al.*, 1988), was offset from the spectral sensitivity curve by 20 nm.

Chromatic adaptation experiments indicate that only one visual pigment is present, as there were no selective effects of green and violet adaptation on the shape of the spectral sensitivity function: spectral sensitivity decreased uniformly across the spectrum (Fig. 2B, C).

The ERG response waveforms in dark-adapted eyes were identical at all wavelengths for individual specimens and adaptation with violet and green lights had no discernible effects on the shape of the response waveforms (Fig. 3). Lack of wavelength-specific effects of

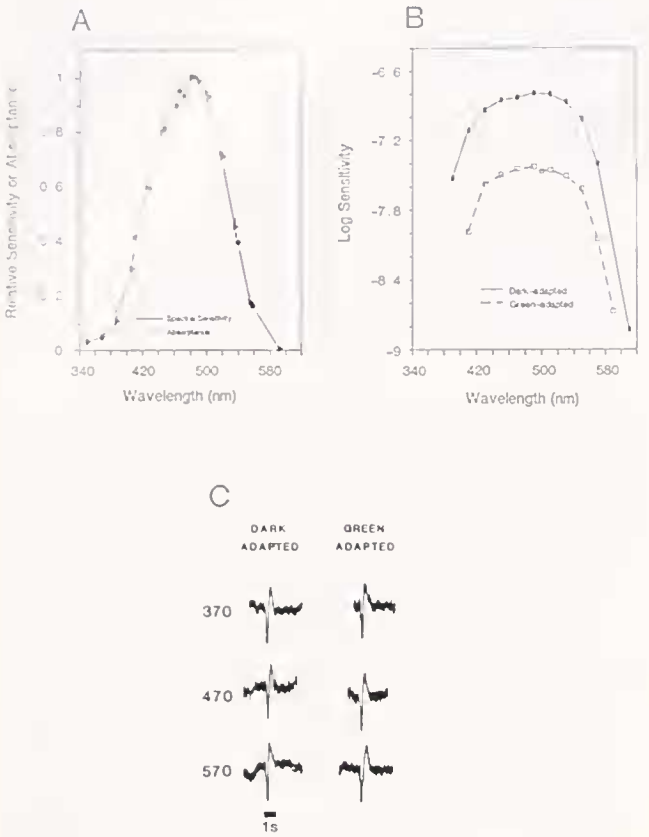

Figure 1. Spectral sensitivity of *Notostomus*. (A) Standardized mean spectral sensitivity curve (solid line) for *N. gibbosus* and *N. elegans* (n = 10). Criterion responses ranged from 20–50 μV. Standard errors are represented by vertical bars. Sensitivity is defined as the reciprocal of the quantum flux (photons/cm^2/s) required to elicit the criterion response at each wavelength. Maximum sensitivity centers on 490 nm. Dashed line represents the absorptance curve for a hypothetical rhodopsin with a λ_{max} of 490 nm, and an O.D. of .5. (B) Green chromatic adaptation had no effect on the spectral sensitivity of *Notostomus* (data from one specimen displayed). Results from four other specimens are the same. Sensitivity is displayed on a log scale so both curves could be displayed on the same axes. Intensity of adaptating light was 1.1 × 10^{-6} μW/cm^2/s. (C) ERG response waveforms, matched for equal amplitude (50 μV), are identical at all wavelengths in the dark-adapted eye, and were not altered by green chromatic adaptation.

chromatic adaptation support the spectral sensitivity evidence that both species possess a single visual pigment.

Systellaspis debilis

Of fifteen *S. debilis* tested, 13 showed heightened sensitivity to violet light. Eight actually possessed two distinct peaks in the dark-adapted spectral sensitivity curves. The variation in the relative sizes of the two peaks (possibly due to variability in electrode location) and the absence of the heightened short wavelength sensitivity in two individuals (Fig. 4) made it inadvisable to combine all the dark-adapted data into one averaged curve. However, the location of the two sensitivity maxima at 400 and 500 nm, when present, was very consistent, as seen in the average curve for the eight specimens in which two maxima were present (Fig. 5A). Spectral sensitivity did

Table I

Depth distribution and bioluminescence mode

Species	Depth range (m)[1] Day	Night	Bioluminescence
Acanthephyra smithi	500–900	200–300	spew
Acanthephyra curtirostris	500–1200	500–1200	spew
Notostomus gibbosus	>900	>900[2]	spew
Notostomus elegans	>900	>900[2]	spew
Systellaspis debilis	620–900	100–300	spew photophores
Janicella spinacauda	500–600	30–250	spew photophores
Oplophorus spinosus	490–750	140–375	spew photophores
Oplophorus gracilirostris	490–650	60–750	spew photophores

[1] Zieman, 1975.
[2] J. Childress, pers. comm.

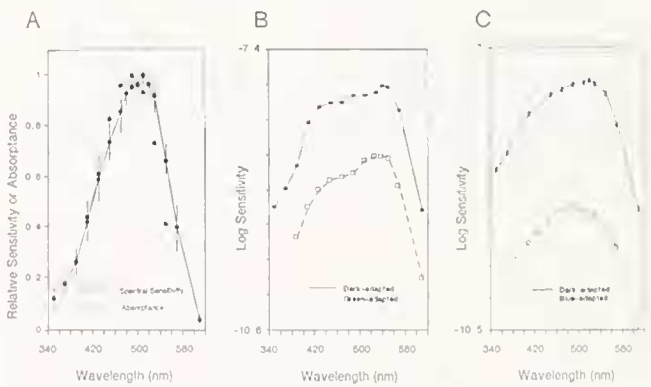

Figure 2. Spectral sensitivity of *Acanthephyra*. (A) Standardized mean spectral sensitivity curve for *A. curtirostris* and *A. smithi* (n = 14). Criterion responses ranged from 20–60 μV. Peak sensitivity centered on 510 nm. Absorptance spectrum (dashed line), was constructed based on the known absorption maximum (490 nm) and O.D. (.6) of *A. smithi* visual pigment (Hiller-Adams *et al.*, 1988). (B) Green chromatic adaptation did not diminish long wavelength sensitivity with respect to short wavelength sensitivity (data from one specimen). Results from three other specimens are the same. Intensity of adapting light was 3.2 × 10^{-5} μW/cm^2/s. (C) Similarly, violet chromatic adaptation did not enhance long wavelength sensitivity relative to short wavelength sensitivity (data from one specimen). Intensity of adapting light was 2.4 · 10^{-4} μW/cm^2/s.

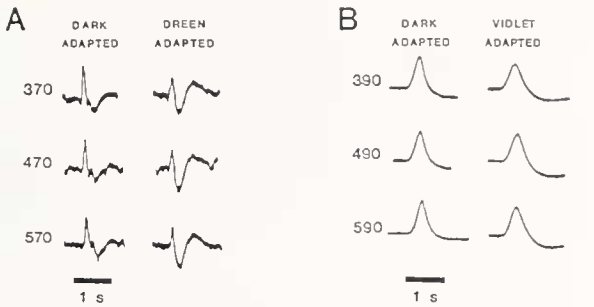

Figure 3. ERG response waveforms matched for equal amplitude (50 μV) in dark-adapted and chromatically adapted *Acanthephyra* (A) Waveforms were identical across the spectrum in the dark-adapted eye. Upon green-adaptation, the responses were different from those in the dark-adapted eye, but were identical to each other. (B) Response waveforms from another dark-adapted specimen were also identical across the spectrum and adaptation with violet light did not affect their shape.

not appear to depend on the size of the criterion response for the range of criterion responses used (20–100 μV), as curves generated for an animal using two different criterion response levels were the same (Fig. 5B, C).

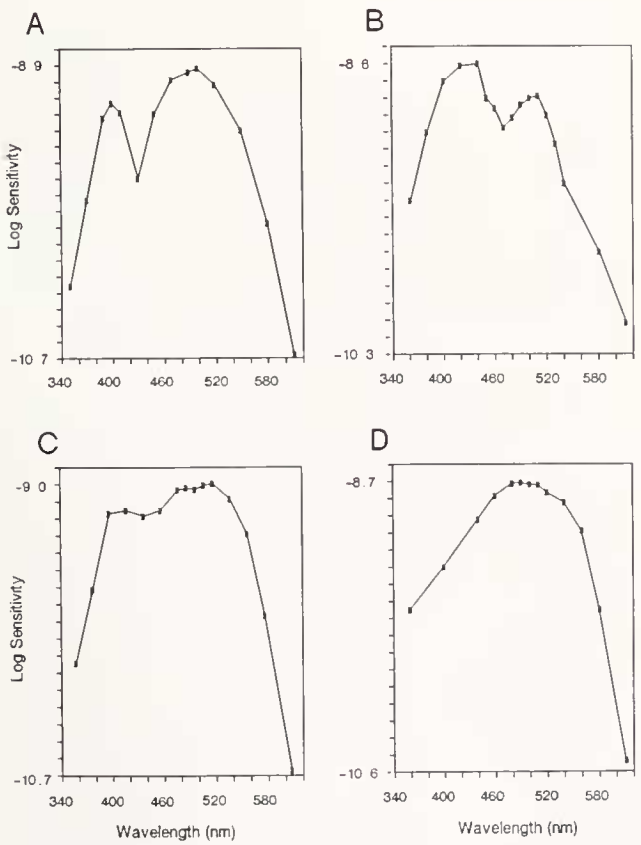

Figure 4. Dark-adapted spectral sensitivity curves from four specimens of *Systellaspis debilis*, demonstrating the variability in their spectral sensitivity. Criterion response = 50 μV.

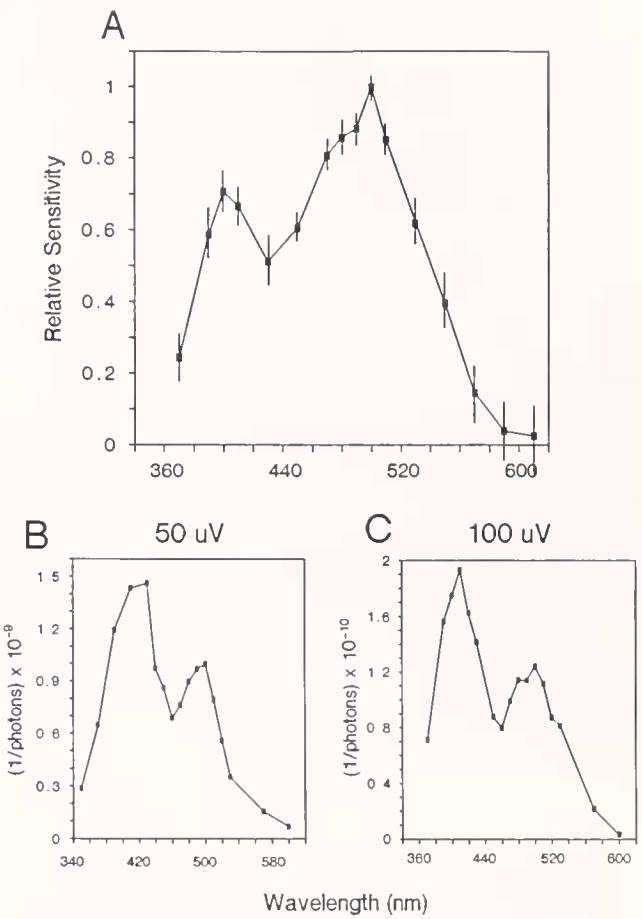

Figure 5. (A) Average standardized spectral sensitivity curve from only those dark-adapted *S. debilis* that possessed bimodal spectral sensitivity curves (n = 8). The two sensitivity maxima were consistently at 400 and 500 nm. (B, C) Dark-adapted spectral sensitivity curves for one preparation at two different criterion response levels were identical.

Results of chromatic adaptation experiments indicate that two visual pigments may be present. Under green adaptation, the spectral sensitivity curve was markedly depressed in the long wavelength part of the spectrum (Fig. 6A). Green adaptation also brought out the violet peaks in two specimens where there was no evidence of a short wavelength peak in the dark-adapted eye (Fig. 6B). The effect of violet adaptation was to depress the short wavelength peak with respect to the long wavelength peak, although the effects were not equally distinct in all experiments. The strongest effects were seen in those specimens that had large 400 nm peaks in the dark-adapted spectral sensitivity curves (Fig. 6C).

Differences in waveform responses to short *versus* long wavelength light suggest that the two putative pigments are in separate cells. Again, because of variability in electrode placement, the dark-adapted waveforms were not identical from animal to animal. In one specimen, the short wavelength response waveforms were distinctly

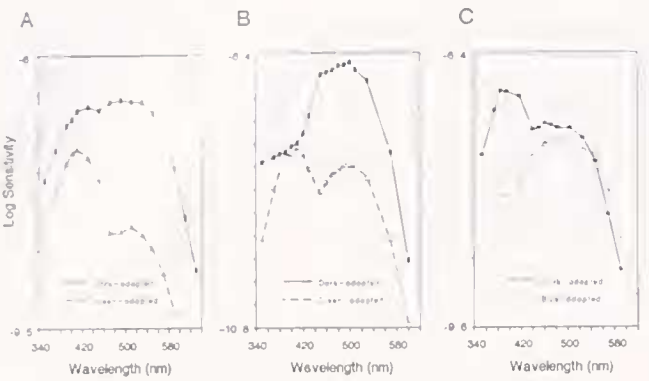

Figure 6. Effects of chromatic adaptation on the spectral sensitivity of *S. debilis*. (A) Green chromatic adaptation had a greater effect on the sensitivity of the blue-green receptors than the violet receptors, leading to a relative enhancement of the violet peak. (B) The dark-adapted spectral sensitivity curve for another preparation showed no distinct violet peak. Green adaptation depressed the sensitivity of the blue-green receptors, exposing the violet receptors and thereby producing a distinct violet peak in the spectral sensitivity function. (C) Blue adaptation had a greater effect on short wavelength sensitivity, depressing the sensitivity of the violet receptors to such an extent that only the blue-green peak is now visible. Results of four other green adaptation and four other blue adaptation experiments were consistent with the results shown. Intensities of adapting lights were (A) 1.2×10^{-4}, (B) 1.1×10^{-5}, and (C) 1.2×10^{-5} $\mu W/cm^2/s$.

different from the long wavelength responses in the dark-adapted eye (Fig. 7A). For another specimen, the waveforms were identical in the dark-adapted eye, but upon green chromatic adaptation, the short wavelength responses became markedly different from the long wavelength responses (Fig. 7B). Blue adaptation had either no effect when waveforms were identical in the dark-adapted eye, or actually diminished differences that were initially present in the dark adapted eye (Fig. 7C).

All of these results support the conclusion that *Systellaspis* possesses two spectral classes of receptor cells with different response characteristics; one with maximal blue-green sensitivity and the other maximally sensitive in the violet.

Janicella spinacauda

The dark-adapted spectral sensitivity curves of the four specimens tested displayed a consistent maximum at 500 nm in the blue-green, but the position of the short wavelength peak varied from 350 to 420 nm (Fig. 8A, B). No correlation could be found between these results and time of capture or time of experimentation.

The results of two chromatic adaptation experiments indicate that two visual pigments are present. The effect of green adaptation was to depress the blue-green peak with respect to the violet peak, as well as shift the short wavelength maximum from 350 to 410 nm (Fig. 8C). Violet adaptation selectively depressed the violet peak relative to the blue-green peak (Fig. 8D).

As in *Systellaspis*, the response waveforms were either distinctly different in the dark-adapted eye (Fig. 9A), or were identical in the dark-adapted state, and changed dramatically at the shorter wavelengths upon green adaptation (Fig. 9B). Conversely, violet chromatic adaptation had no selective effects on response waveforms that were identical in the dark-adapted eye (Fig. 9C).

These results indicate that *Janicella* also possesses two spectral classes of receptor cells.

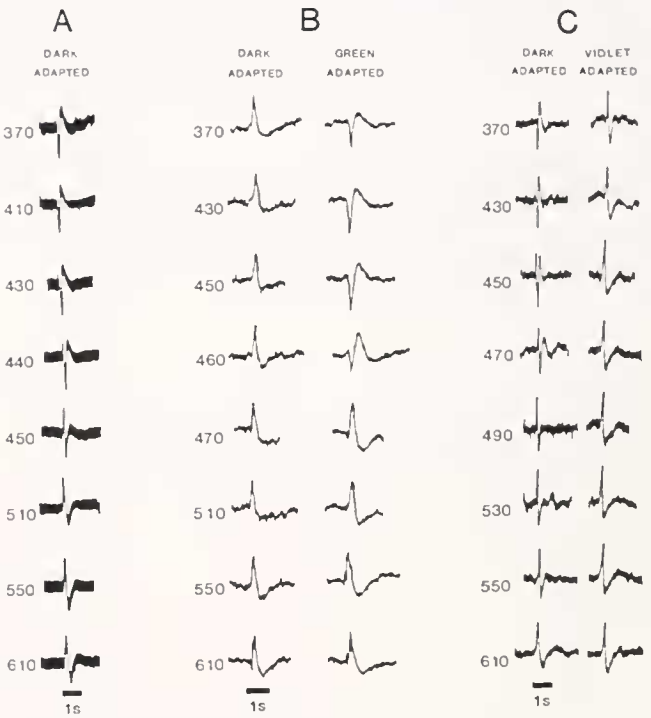

Figure 7. Effects of chromatic adaptation on the response waveforms matched for equal amplitudes in *S. debilis*. (A) Response waveforms of this specimen (criterion response = 40 μV) were distinctly different at different wavelengths. From 370 to 410 nm, the main component of the ERG was corneal positive (downward). Between 430 and 440 nm, a small corneal negative component preceded the larger positive portion. From 450 nm to 610 nm, the main component was corneal negative (upward). (B) Response waveforms (30 μV) in another preparation were identical at all wavelengths in the dark-adapted eye, with simple, monophasic, corneal negative waveforms. Green adaptation produced distinct wavelength specific effects. The waveforms from 350 to 450 nm were reversed in polarity, while the responses from 470 to 610 nm remained unchanged, with the transition occurring at 460 nm. (C) Dark-adapted response waveforms (50 μV) demonstrate the same differences as described in (A), with waveforms between 370 and 430 nm exhibiting one characteristic waveform, and responses between 470 and 610 nm exhibiting another. The transition from one type to the other occurred at 450 nm. Blue adaptation markedly altered the response waveforms between 370–450 nm; these waveforms became identical to the long wavelength responses, which were unaffected by blue adaptation.

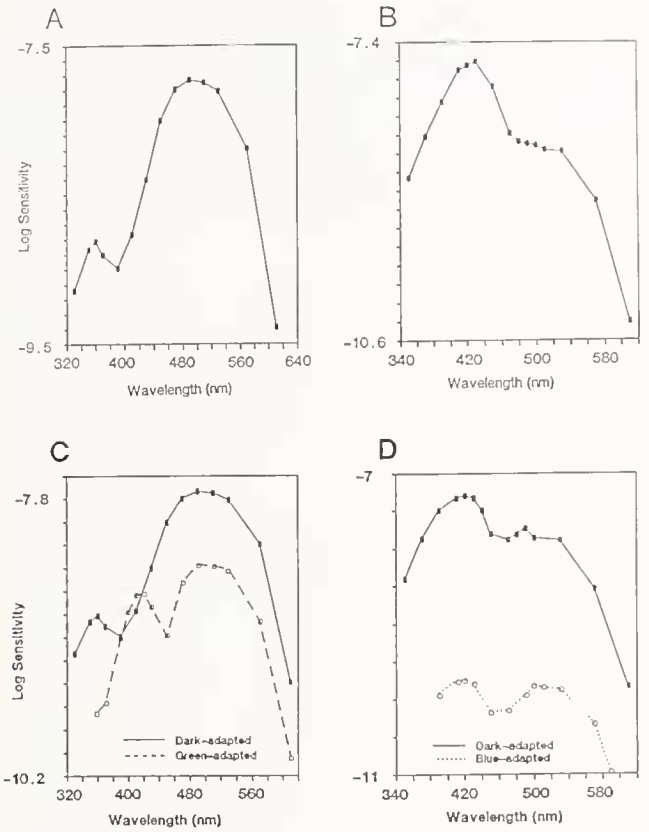

Figure 8. Dark-adapted spectral sensitivity curves for individual specimens of *Janicella spinacauda*. (A) The short wavelength sensitivity peaks at about 350 nm, while the long wavelength sensitivity peaks at 500 nm. (B) In another specimen, the short wavelength peak was at 420 nm, while the long wavelength sensitivity maximum was a shoulder rather than a distinct peak. (C) Green chromatic adaptation enhanced the relative size of the violet peak, as well as shifting the sensitivity maximum from 350 to 410 nm. Data from one specimen. Intensity of adapting light was 1.56×10^{-6} $\mu W/cm^2/s$. (D) Violet chromatic adaptation had a greater effect on short wavelength sensitivity, resulting in a relative enhancement of the blue-green peak. Data from one specimen. Intensity of adapting light was 1.2×10^{-3} $\mu W/cm^2/s$.

Oplophorus spinosus *and* O. gracilirostris

The results for *O. spinosus* and *O. gracilirostris* were the same, and will be discussed together with no distinction between species. Representative examples of dark-adapted spectral sensitivity curves for two specimens are shown in Figure 10 (A, B). The variability in these curves is similar to that seen in the previous two species.

Chromatic adaptation experiments again provide evidence that more than one visual pigment is present. Violet adaptation resulted in a small depression in the violet shoulder (Fig. 10C). The only specimen that had a distinct violet peak in its dark-adapted spectral sensitivity curve (see Fig. 10B) died during the chromatic adaptation experiment; therefore, the effects of violet adaptation are not as apparent as in *Systellaspis* or *Janicella*. The effects of green adaptation were much more dramatic. The sensitivity to long wavelength light was greatly diminished with respect to the short wavelength sensitivity, resulting in either two peaks, or, with more intense adaptation, a distinct peak at 400–410 nm, and a plateau centering at 500 nm (Fig. 10D).

The shapes of the response waveforms in dark-adapted and chromatically adapted eyes were the same as those described for *Systellaspis* and *Janicella* (Fig. 11C), again pointing to the presence of two spectral classes of receptor cells.

Oplophorus spinosus proved to be unusually robust, and in two instances we were able to record responses after the eye had recovered from chromatic adaptation. Green chromatic adaptation distinctly altered the shape of the spectral sensitivity curve as well as the response waveforms (Fig. 11A, C). Both the spectral sensitivity curve and the response waveforms, measured two hours after extinguishing the adapting light, were the same as those measured before chromatic adaptation (Fig. 11B, C). This indicates that waveform changes were due to the effects of the adapting light, and not to changes in electrode position or to degenerative changes in the eye during the course of an experiment.

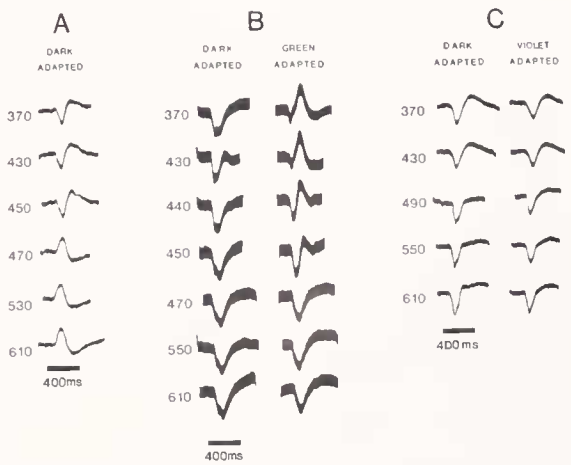

Figure 9. ERG response waveforms matched for equal amplitude in *J. spinacauda*. (A) The response waveforms (amplitude = 30 μV) in this dark-adapted preparation were markedly different between responses to short *versus* long wavelength light. The major component of the short wavelength responses (350–450 nm) was corneal positive (shown by the downward deflection), while the major component of the longer wavelength responses was negative. (B) In another preparation, the dark-adapted response waveforms (40 μV) were virtually identical, and were all corneal positive. Upon adaptation with green light, the waveforms between 370–450 nm were reversed in polarity, while the waveforms from 470–570 nm remained unchanged. (C) Dark-adapted waveforms in another preparation were virtually identical, and remain unchanged under a blue adapting light. Polarity differences in long wavelength response waveforms between different specimens are probably due to differences in the depth of the recording electrode (see Discussion).

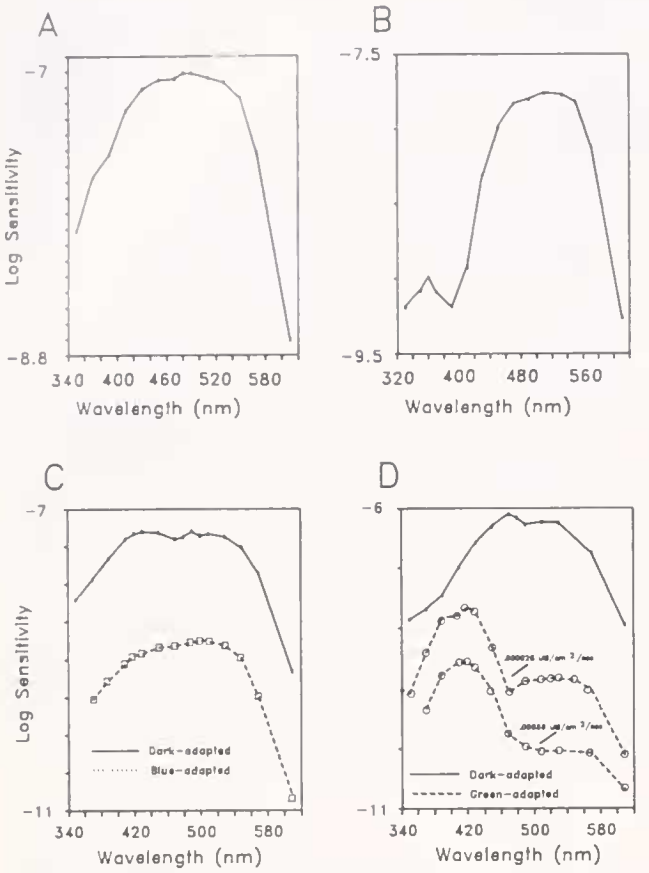

Figure 10. Dark-adapted spectral sensitivity curves for *Oplophorus*. (A) Six of the seven specimens tested possessed broad spectral sensitivity curves similar to the one shown, with small variations in sensitivity at the shorter wavelengths. (B) Only one distinctly bimodal spectral sensitivity curve was measured, with peaks at 350 and 500 nm. (C) Selective effects of violet adaptation are small but discernible; sensitivity was slightly more depressed at the shorter wavelengths, diminishing the small violet peak seen in the dark-adapted eye. Intensity of adapting light was 2.4×10^{-4} $\mu W/cm^2/s$. (D) Green adaptation selectively depressed sensitivity at the longer wavelengths, producing a much larger violet peak relative to the blue-green peak. Under a higher intensity adapting light, the blue-green peak was completely depressed in the same specimen. Results from four other specimens are compatible with those shown.

Discussion

In clear oceanic waters, the wavelength of maximum light transmittance is 510 nm in the surface layers, with the FWHM covering a spectral range from 440 to 600 nm. At 100 m depth, selective absorption and scattering have shifted the transmission maximum to 475 nm and narrowed the spectral distribution to a FWHM covering 440–500 nm (Jerlov, 1968; Dartnall, 1974; Jerlov, 1976; Cronin, 1986). The possibility that deep-sea organisms may have blue-shifted visual pigments as an adaptation for maximum sensitivity to this light regime (the Sensitivity Hypothesis) was first suggested by Clarke (1936) and Bayliss *et al.* (1936), and this idea of sensitivity peaks matching ambient light distribution has since been extended to other environments. Although Lythgoe (1968) has shown that the Sensitivity Hypothesis may not necessarily hold true for all shallow water species, which live in a very "complex" visual environment, it has been strongly supported by studies on organisms living in the

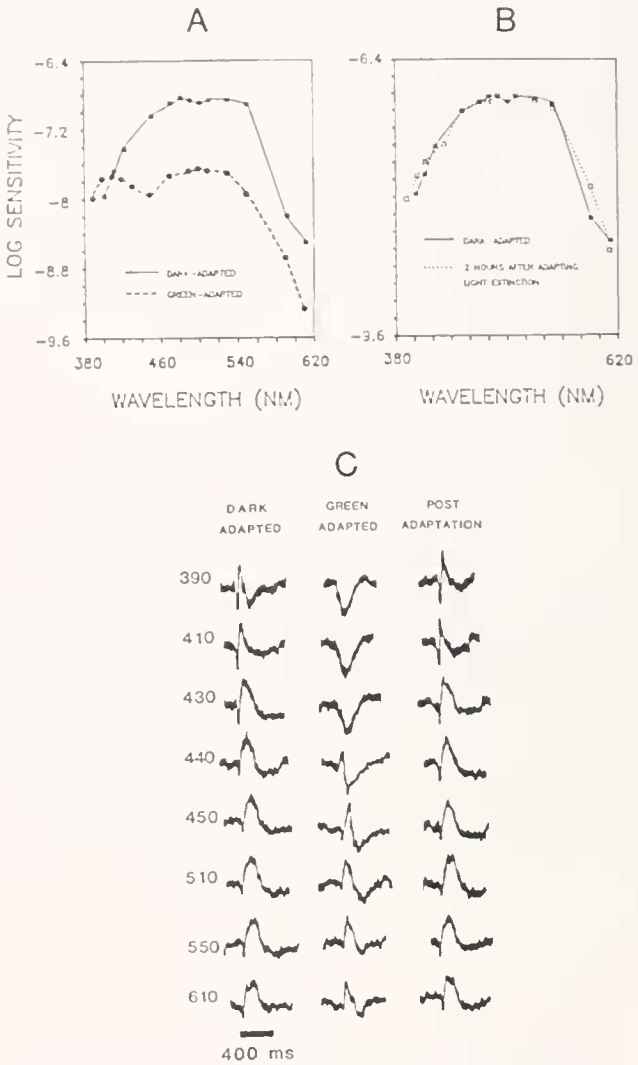

Figure 11. Effects of chromatic adaptation on spectral sensitivity and response waveforms in *Oplophorus*. (A) Green adaptation selectively depressed the long wavelength sensitivity, leading to a bimodal spectral sensitivity curve with maxima at 400 and 500 nm. (B) Two hours after the adapting light was turned off, the eye had recovered completely from the effects of the adapting light, and the spectral sensitivity function was identical to that of the dark-adapted eye. (C) Dark-adapted response waveforms for the same preparation shown above were slightly different at the shortest wavelengths. Green adaptation altered the response waveforms at the shorter wavelengths as previously described. Two hours after the adapting light was turned off, the response waveforms were again identical to those in the dark-adapted eye. Adapting light intensity = 2.5×10^{-4} $\mu W/cm^2/s$.; criterion response = 40 μV.

"simpler" deep-sea visual environment. The visual pigments of most deep-sea species studied to date have peak absorption maxima clustered between 470 and 490 nm, which are about 10–20 nm shorter than those of their shallow water counterparts, thus supporting the Sensitivity Hypothesis (reviewed by Goldsmith, 1972; Cronin, 1986).

Single visual pigment systems

The results from two species in this study, *N. gibbosus* and *N. elegans*, support the Sensitivity Hypothesis. The maximum sensitivity of these species (490 nm) is at shorter wavelengths than those of shallow water crustaceans (510–550 nm), and fall into the same range as those of the deep-sea fish (Denton and Warren, 1957; Munz, 1957; Wald *et al.*, 1957; Denton and Shaw, 1963; Fernandez, 1978; Crescitelli *et al.*, 1985).

The spectral sensitivities of *A. curtirostris* and *A. smithi* peak at 510 nm, seemingly more appropriate for shallow water crustaceans than for species that maintain daytime depths of greater than 500 m. The absorptance spectrum matches the shape of the spectral sensitivity curve, but is offset by 20 nm. This suggests that these species may possess some type of non-moving distal pigment screen, as found in *A. purpurea* (Welsh and Chace, 1937), that would shift the sensitivity maximum away from the visual pigment absorption maximum. In crayfish and lobsters, this pigment screen is believed to be responsible for the 10–30 nm difference between the visual pigment absorption and the spectral sensitivity function (Goldsmith, 1978). Why such a screening pigment shield would be needed, particularly in *A. curtirostris*, which never migrates to shallower waters, remains obscure.

It is unlikely that self-screening by metarhodopsin contributed significantly to the long wavelength shift in spectral sensitivity. Although both species possess metarhodopsins with λ_{max} at shorter wavelengths (*A. curtirostris*—481 nm; *A. smithi*—483 nm; Hiller-Adams *et al.*, 1988) than those of their rhodopsins, so that self-screening by metarhodopsin would shift the spectral sensitivity to longer wavelengths, our experimental protocol ensured that the eye was fully dark-adapted before starting an experiment. According to Goldsmith (1978), self-screening by metarhodopsin should be negligible if: (1) the eye is dark-adapted, (2) near-threshold flashes are used to stimulate the eye (preventing isomerization of a sizable fraction of rhodopsin to metarhodopsin), and (3) the organism has other mechanisms than photo-regeneration for restoring a full titer of rhodopsin. The first two conditions were met by our experimental protocol, and while these two species have not been studied with respect to dark-regeneration, such a system was found in another oplophorid occupying the same depth range (Hiller-Adams *et al.*, 1988.) Additionally, specimens tested within three hours of capture demonstrated the same spectral sensitivity as those that were maintained in the dark for 24 hours before testing. Therefore, self-screening by metarhodopsin is not a reasonable explanation for the observed results.

The difference in the polarities of the representative response waveforms shown for *Notostomus* (Fig. 1) and *Acanthephyra* (Fig. 3) may be due to differences in the electrode depths from preparation to preparation. In both genera, preparations were found in which the response waveforms were of the opposite polarity to those shown in the figures, so these polarity differences are not species specific, but probably depend on the recording parameters. Konishi (1955), working with the lobster eye, showed that an electrode just beneath the corneal surface recorded a corneal negative response. With deeper insertion into the eye, the recorded response reversed in polarity to a corneal positive response. Since the depth of electrode penetration varied between preparations in our study, electrode position may explain the ERG polarity differences.

Dual visual pigment systems

The most interesting visual systems are found in the remaining four species, *S. debilis*, *J. spinacauda*, *O. spinosus*, and *O. gracilirostris*, which appear to possess a violet sensitive pigment in addition to one with maximal sensitivity in the blue-green. The variation in the shapes of the dark-adapted spectral sensitivity curves is much greater than in those of the single pigment species, and this may be due in part to the location of the electrode in the eye, particularly if different parts of the eye have different spectral sensitivities. Regional differences in spectral sensitivity have been found in the eyes of several species of insects (Walther, 1958; Ruck, 1965; Bennett and Ruck, 1970) and results of experiments on these insects are similar to ours. Goldsmith (1960) also reports that the relative contribution of the UV and green receptor systems to the ERG in the honeybee eye could be altered somewhat by moving the electrode to another part of the eye.

Due to our experimental protocol in testing the eye with a standard flash throughout the experiment, in addition to the fact that those crustaceans which had a distinct violet peak exhibited the same overall sensitivity as those which did not (see Fig. 4), we are confident that the differences in shapes of dark-adapted spectral sensitivity curves were not due to differences in the degree of dark-adaptation.

The conclusion that two visual pigments are present in these four species is strongly supported by the differential

effects of the different adapting lights on the shape of the spectral sensitivity curve. The selective effects of violet adaptation were generally not as great as those of green adaptation, and this can be attributed to the fact that all visual pigments possess a β-band that absorbs in the shorter wavelengths, meaning that violet adaptation would affect both receptor systems. However, the fact that violet adaptation had a stronger effect on the short wavelength system, so that differential effects in the shape of the spectral sensitivity functions could be seen, indicates that the violet peak is not due to the β-absorption band on the blue-green pigment as in the woodlouse *Porcellio* (Goldsmith and Fernandez, 1968). If this were the case, the sizes of the two peaks relative to each other would remain the same during all adaptation conditions.

Waveform differences between responses to short *versus* long wavelength light indicate that the two visual pigments are housed in different receptor cells. Single cells have never been shown to respond differentially to different wavelengths of light (Graham and Hartline, 1935; Naka and Rushton, 1966; Stark and Wasserman, 1974), and if several spectral mechanisms with different time courses contribute to the ERG, equal amplitude responses at all wavelengths can never be matched (Chapman and Lall, 1967). In several species of muscid flies, waveform differences between short and long wavelength responses were initially attributed to the presence of a red sensitive receptor in addition to short wavelength receptors (Autrum and Burkhardt, 1961; Burkhardt, 1962; Mazokhin-Porshnyakov, 1960). However, Goldsmith (1965) found that these differences were due to differences in the sizes of ganglionic on-off effects in the ERG, rather than the presence of several spectral classes of receptor cells. Crustacean ERGS do not exhibit these ganglionic on/off effects, since the ERG is a more purely retinal response (Naka and Kuwabara, 1956; Chapman and Lall, 1967; Goldsmith and Fernandez, 1968), and there is no experimental evidence for any contribution by the optic ganglion to the ERG (Ruck and Jahn, 1954; Konishi, 1955). Therefore, differences in waveform responses to short and long wavelength light in crustaceans, based on current knowledge, can only be attributed to two different populations of receptor cells with different membrane properties (Wald, 1968).

Further support for two spectral classes of receptor cells comes from the wavelength-specific effects of different adapting lights on response waveforms. These results can be explained by assuming that the visual pigments are housed in different receptor cells, and that the numeric distribution of the two receptor classes is not equal. This situation is found in crayfish and lobsters, where the long wavelength pigment occupies seven of the eight retinula cells present in each ommatidium, and the violet pigment occupies just one (Cummins and Goldsmith, 1981; Cummins *et al.*, 1984). A similar unequal distribution also appears to be present in our deep-sea species. The ERG responses in the dark-adapted eyes were generally characteristic of those attributed to the blue-green receptors. Upon green adaptation, the relative contribution of the violet receptors was enhanced, and distinct differences in response waveforms at the shorter wavelengths were observed. Conversely, violet adaptation had no affect on response waveforms, or diminished differences present at the shorter wavelengths in the dark-adapted eyes, as expected when the minor contribution of the violet receptors was further diminished.

The location of the violet receptor cells in an accessory eye would provide an explanation for the unusual "hyperpolarizing" responses seen to short wavelength light. All known microvillar photoreceptors, which are the type possessed by all crustaceans (Eakin, 1972), depolarize in response to light (reviewed by Jarvilehto, 1979), but an unusual orientation of the short wavelength receptors to the electrode could produce an apparently "hyperpolarizing" response. In the aleiopid worm *Torrea*, which also has microvillar photoreceptors, responses from the main retina are depolarizing, while responses contributed by an accessory retina are hyperpolarizing, and this has been attributed to the reversed arrangement of the receptors of the accessory retina relative to the electrode position (Wald and Rayport, 1977).

Functions of two visual pigments in deep-sea organisms

Many other crustaceans, such as shallow water crabs (Wald, 1968; Hyatt, 1975; Martin and Mote, 1982), lobsters (Cummins *et al.*, 1984), estuarine shrimp (Wald and Seldin, 1968; Goldsmith and Fernandez, 1968), and crayfish (Goldsmith and Fernandez, 1968; Wald, 1968; Waterman and Fernandez, 1970; Cummins and Goldsmith, 1981) appear to possess a short wavelength visual pigment. The purpose of this pigment is not clear, although Hyatt (1975) feels that it may be a mechanism for hue discrimination in the fiddler crab. Even though the rationale for the pigment is not known, all of these species live in shallow or near-surface waters where UV light is abundant and may play some role in their visual environment. In insects, the presence of a UV peak has been closely correlated with some behavioral patterns. Behavioral studies on UV-sensitive pierid butterflies indicate that they visit violet and blue flowers more often than butterflies without the short wavelength sensitivity (Ilse, 1928; Eguchi *et al.*, 1982). Obara and Hidaka (1968) also report that male pierid butterflies approach females after identifying the UV patterns on their wings. However, the reason for a UV visual pigment among some nocturnal moths (Eguchi *et al.*, 1982; Mikkola,

1972) remains obscure, since UV light is absent in moonlight as well as in background galactic light at night (Munz and McFarland, 1973, 1977).

There is a similar absence of UV and near-UV light in the deep-sea. Although UV light may penetrate significantly in the surface layers (Jerlov, 1968; Dartnall, 1974; Jerlov, 1976), it is virtually absent by 500 meters—.09% of the 500 nm light present at the surface remains, while only .00007% of the 400 nm light is still present (Type 1 water, Table XXVI—Jerlov, 1976).

Bioluminescence, the other source of light in the deep-sea, is considered by some to be the major visual stimulus present in this environment (Beebe, 1935; Clarke and Hubbard, 1959; Jerlov, 1968). It is also the logical candidate to provide an explanation for the violet visual pigment, since the four species with the enhanced violet sensitivity possess photophores, while the four species without photophores are not violet sensitive. Examples of unusual visual systems correlated with bioluminescence are found in three species of malacosteid fish, which possess red-shifted visual pigments as an apparent adaptation for enhanced sensitivity to their own red bioluminescence (O'Day and Fernandez, 1974; Denton *et al.*, 1970; Bowmaker and Herring, unpub.). While the vast majority of bioluminescence emission maxima in the deep-sea, including those from the photophores of *S. debilis* and *O. spinosus*, are clustered around the same wavelengths as the downwelling illumination (460–490 nm), with no emission maxima below 430 nm (Herring, 1976; Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988), bioluminescence may still provide the explanation for the unusual violet pigment.

It has been suggested that the presence of two blue-green visual pigments in some species of deep-sea fish may serve as a system for discriminating between different bioluminescent organisms by using the spectral bandwidth as the basis for discrimination (Partridge *et al.*, 1988). The putative violet visual pigment may be serving the same purpose in these oplophorids. Spectra with broader spectral bandwidths would be more efficient in stimulating the violet-receptor, and in this manner could be distinguished from spectra with narrower bandwidths. The spectral emissions from most of the species with similar depth distributions as these oplophorids, including other crustaceans (except euphausiids), siphonophores, fish, and cephalopods, are remarkably similar, with the peaks lying between 465 and 485 nm, and FWHM covering 65–90 nm (Widder *et al.*, 1983; Herring, 1983; Latz *et al.*, 1988). However, the emissions from the photophores of *S. debilis* and *O. spinosus*, while peaking in the same range, have FWHMs of 48–58 nm (Herring, 1983; Latz *et al.*, 1988), and perhaps this difference in spectral bandwidth is enough to facilitate congener recognition. Additionally, the FWHM of their luminous secretion, which is thought to be used during escape responses, is between 65 and 75 nm, and could potentially be distinguished from the photophore emission to serve as warning signs to congeners.

Acknowledgments

We thank Dr. James Childress, his laboratory associates, and the captains and crews of the RV *New Horizon* for assistance with animal collection. We also thank Dr. Childress for generously providing shipboard laboratory space; Dr. Steve Bernstein for writing the digitizing program; and Mark Lowenstine, Robert Fletcher, and Joel Dal Pozzo for help in designing and building the portable ERG apparatus. Drs. Thomas Cronin, Edith Widder, and Michael Latz provided helpful comments and suggestions. This work was supported by a grant from the Office of Naval Research (N00014-84-K-0314) to J. F. Case, a National Science Foundation grant (OCE 85-000237) to J. J. Childress, and a UCSB patent fund grant and dissertation fellowship to T. M. Frank.

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